solutions of mTFP-PNA monomer units resulted in template directed induced assembly. Assembly was confirmed by SDS-PAGE, mass spectrometry, and size exclusion HPLC.
Fluorescence anisotropy was monitored over the course of a mTFP-PNA:DNA titration. Template coding for dimer formation was studied. The anisotropy showed a decreasing trend related to homo-FRET in the assembled forms. A maximal reduction (41%) was observed at a DNA to mTFP-PNA ratio of 1:2. Anisotropy, then increased steadily up to a 1:1 ratio.
This study demonstrates an inducibly assembled homo-FRET system using expressed protein ligation which may be extended to study oligomerization and cluster formation in living systems.

2025-Pos Board B44
Effect of Lipid Bilayers on Prion Peptide Aggregation: Insights from Coarse-Grained Molecular Simulations
Mikyung Seo, D. Peter Tieleman.
University of Calgary, Calgary, AB, Canada.
Prion diseases are neurodegenerative diseases associated with a conformational change of the normal cellular form of the prion protein (PrP^{C}) to an abnormal aggregated form (PrP^{Sc}). Recent research suggests that oligomeric rather than plague forms of prion protein (PrP) are the main toxic species, but it is not clear how they lead to disease development. However, the interactions of PrP with membranes have been reported to affect the behavior of PrP, and have been implicated in the toxicity of oligomers. To gain insight into the molecular basis of this effect, we use coarse-grained molecular simulations to study the ability of several amyloidogenic PrP and yeast prion protein fragments to interact with zwitterionic and anionic model membranes. Monomeric and oligomeric forms of PrP are studied in water and in the presence of phosphatidylcholine (POPC) and phosphatidylserine (POPS) bilayers. The conformation of PrP and peptide-lipid interactions are characterized, and the influence of peptide binding to different lipid bilayers on the aggregation process is analyzed.

2026-Pos Board B45
Pores Versus Fibrils: Calcium Ions Regulate Different IAPP-Mediated Membrane Damage Mechanisms
Michele F. Sciacca[1], Danilo Milardi[2], Grazia M.L. Messina[3], Giovanni Marletta[3], Jeffrey R. Brender[1], Ayyalusamy Ramamoorthy[1], Carmelo La Rosa[3].
[1]University of Michigan, Ann Arbor, MI, USA, [2]Istituto di Biostrutture e Bioimmagini CNR-UOS, Catania, Italy, [3]University of Catania, Catania, Italy.
The disruption of plasma membrane integrity by amyloidogenic proteins is linked to the pathogenesis of a number of common, and frequently deadly, degenerative diseases. using hIAPP (an amyloidogenic peptide associated with beta-cell death in type II diabetes) as an example, we demonstrate that Ca^{2+} ions modulate the membrane interaction of hIAPP, significantly enhancing fiber-dependent membrane disruption while suppressing a pore-like mechanism. QCM, AFM, and NMR results show that Ca^{2+} ions promote a shallow membrane insertion of hIAPP, which leads to both the early accumulation of non-fibrillar oligomers on the membrane surface and later detergent-like removal of lipids from the bilayer triggered by fiber growth. Since both mechanisms are common to amyloid toxicity by most amyloidogenic proteins, it is likely that unregulated Ca^{2+} homeostasis, amyloid aggregation, peptide binding to lipids and membrane leakage are all parts of a self-perpetuating cycle fueling amyloid cytotoxicity.

2027-Pos Board B46
Specific Sequences within Beta-Amyloid Mediate Aggregation Associated with Lipid Membranes
Elizabeth A. Yates, Sherry L. Finkel, Michael Lynch, Elena M. Cucco, Charles S. Umbaugh, Justin Legleiter.
West Virginia University, Morgantown, WV, USA.
A hallmark of Alzheimer's disease (AD), a late onset neurodegenerative disease, is the presence of neuritic amyloid plaques deposited within the brain comprised of beta-amyloid (Aß) peptide aggregates. Aß forms a variety of nanoscale, toxic aggregates which have been shown to strongly interact with supported lipid bilayers, which may represent a key step in potential toxic mechanisms. Understanding how specific regions of Aß regulate its aggregation in the absence and presence of surfaces can provide insight into the fundamental interaction of Aß with cellular surfaces. We investigated the interaction of specific fragments of Aß (Aß1-11, Aß1-28, Abeta10-26, Aß12-24, Aß16-22, Aß22-35, and Aß1-40) with lipid membranes. These sequences represent a variety of chemically unique regions along Aß, i.e., the extracellular domain, the central hydrophobic core, and transmembrane domain. We determined how these Aß sequences alter aggregate morphology and induce mechanical changes of lipid bilayers using various scanning probe microscopic techniques, and compared these aggregates with those formed under free solution conditions. In free solution, oligomer and fibrillar aggregate species were formed with varied rate of formation and morphology, i.e. smaller fragments (Aß1-11, Aß12-24, Aß16-22, and Aß22-35) formed smaller oligomers, and shorter, less rigid fibrils. Interaction with model lipid bilayers resulted in distinct aggregates and changes in bilayer stability dependent on the Aß fragment. Aß10-26, Aß16-22, Aß22-35, and Aß1-40 caused disruption of the lipid bilayer structure upon exposure and resulted in a variety of distinct fibrillar aggregates. These interactions were associated with altered mechanical properties of the lipid bilayer. Conversely, Aß1-11, Aß1-28, and Aß12-24 had minimal interaction with a lipid membrane, forming only oligomers. These studies illustrate the potential role of specific amino acid sequences within Aß on aggregation and interactions with lipid membranes.

2028-Pos Board B47
The Interactin of Huntingtin Exon1 with Lipid Bilayers is Regulated by polyQ Length and polyQ Flanking Sequences
Kathleen Burke, Charles S. Umbaugh, Kaitlin Hensal, Justin Legleiter.
West Virginia University, Morgantown, WV, USA.
Huntington's Disease (HD) is a neurodegenerative disorder that is defined by the accumulation of nanoscale aggregates comprised of the huntingtin (htt) protein. Aggregation is directly caused by an expanded polyglutamine (polyQ) domain near the N-terminus of htt, leading to a diverse population of aggregate species, including oligomers and fibrils. Furthermore, the length of the polyQ domain is directly related to onset and severity of disease. The first 17 amino acids on the N-terminus (N17) and the polyproline (polyP) domain on the C-terminal side of the polyQ domain have been shown to further modulate the aggregation process. Additionally, N17 appears to have lipid binding properties as htt interacts with a variety of membrane-containing structures present in cells, such as organelles, and interactions with these membrane surfaces may further modulate htt aggregation. To investigate the interaction between htt exon1 and lipid bilayers, in situ atomic force microscopy (AFM) was used to directly monitor the aggregation of htt exon1 constructs with varying polyQ-length or synthetic peptides with different combinations of polyQ domain flanking sequences associated with htt exon1 on supported lipid membranes comprised of total brain lipid extract. The exon1 fragments accumulated on the lipid membranes, causing disruption of the membrane, in a polyQ-length dependent manner. By adding N-terminal tags to the htt exon1 fragments, the interaction with the lipid bilayer was impeded. Synthetic peptides lacking the N17 flanking sequence had no appreciable interaction with lipid bilayers. Interestingly, polyQ peptides with the N17 flanking sequence interacted with the bilayer. This interaction was further modulated by the addition of the polyP domain.

2029-Pos Board B48
The Molecular Assembly of the Aerolysin Pore Reveals a Unique Swirling Membrane-Insertion Mechanism
Matteo Degiacomi, Ioan Iacovache, Gisou van der Goot, **Matteo Dal Peraro**.
EPFL Lausanne, Lausanne, Switzerland.
Aerolysin is the founding member of a super-family of ß-pore forming toxins for which the pore structure is unknown. We have combined X-ray crystallography, cryo-electron microscopy (EM), molecular dynamics and computational modeling to determine the structures of aerolysin mutants in their monomeric and heptameric forms, trapped at various stages of the pore formation process. A dynamic docking approach based on swarm intelligence was applied whereby the intrinsic flexibility of aerolysin extracted from new X-ray structures was utilized to fully exploit the cryo-EM spatial restraints. using this integrated strategy, we obtained a radically new arrangement of the prepore conformation and a near-atomistic structure of the aerolysin pore, which is fully consistent with all biochemical data available so far. Upon transition from the prepore to pore, the aerolysin heptamer shows a unique concerted swirling movement, accompanied by a vertical collapse of the complex, ultimately leading to the insertion of a transmembrane ß-barrel.

2030-Pos Board B49
Concentration Dependent Transition of Membrane-Bound Beta-Stranded KIGAKI Peptides from Unstructured Monomers into Immobilized Amyloid Fibrils Observed by Solid-State ^{19}F-NMR
Erik Strandberg, Parvesh Wadhwani, Jochen Bürck, Nico Heidenreich, Susanne Fanghänel, Anne S. Ulrich.
Karlsruhe Institute of Technology, Karlsruhe, Germany.
The structure, membrane alignment, flexibility, and aggregation behavior of the β-stranded antimicrobial peptide KIGAKI [with sequence $(KIGAKI)_3$-NH_2] has been determined in oriented lipid bilayers using circular dichroism (CD), oriented CD (OCD), and solid state NMR spectroscopy. Several IIe or Ala residues were replaced one at a time with CF_3-L/D-Bpg or Ala-d_3. At high

peptide-to-lipid molar ratios (P/L) of 1:200 or above, the ^{19}F dipolar coupling of all labeled positions exhibits the maximum possible value of +16 kHz, indicating that at high concentration KIGAKI self-assembles into immobilized β-sheets, which lie flat on the membrane surface, which is also supported by CD and OCD spectra. Transmission electron microscopy images reveal that the aggregated KIGAKI forms amyloid-like fibrils, with less propensity for the CF_3-D-Bpg labeled peptides to aggregate, compared with the CF_3-L-Bpg labeled peptides. This aggregation difference is also reflected in the biological activity of the differently labeled peptides. At low peptide concentrations, on the other hand, all labeled positions show dipolar couplings of +8 kHz, indicating that their time-averaged alignment is still parallel to the bilayer normal, but the mobility of the peptides increases drastically, with monomeric peptides being the most likely state. Thus, flexible β-strands float on the membrane surface and undergo motional averaging in the membrane plane, similar to intrinsically unstructured proteins in solution. This is the first example of a concentration dependent transition of a flexible β-strand to an amyloid-like fibril on membrane surfaces that has been directly observed by solid state NMR.

Protein Folding & Stability I

2031-Pos Board B50

Conversion of the Sulfhydryl Oxidase Augmenter of Liver Regeneration into a Selenoprotein

Stephanie Aron Schaefer, Ming Dong, Brian Bahnson, Colin Thorpe, Sharon Rozovsky.
University of Delaware, Newark, DE, USA.

Augmenter of liver regeneration (ALR) is a flavin-dependent sulfhydryl oxidase with roles in mitochondrial oxidative protein folding and cellular signaling. To study ALR's reaction mechanism we have prepared a form of the enzyme in which sulfur was replaced with selenium. The selenium-rich ALR is catalytically active, thermally stable, and its structure is almost identical to that of the native ALR. The presence of selenium in the active site leads to the formation of a charge-transfer complex during turnover, as detected by visible spectroscopy. To further demonstrate the role selenium plays in ALR's active site, we have utilized E. coli's selenium insertion machinery to introduce selenium in a site-specific manner. using this method we are able to convert ALR's redox active CxxC motif to a selenocysteine containing CxxU motif. Our results demonstrate that the selenocysteine proximal to the FAD cofactor is sufficient to cause a charge-transfer complex during turnover. In addition, 77Se NMR spectroscopy was used to probe locations typically occupied by sulfur - an insensitive nucleus that is not amendable for NMR studies of proteins. Biological 77Se NMR has so far been underutilized due to the challenges of isotopically enriching protein with 77Se. Here, we have developed a method to introduce 77Se by heterologous expression in E. coli. We report the NMR spectra of ALR bound to oxidized and reduced FAD. An unidentified resonance appears only in the presence of the reducing agent and disappears readily upon exposure to air and subsequent reoxidation of the flavin. Hence, 77Se NMR spectroscopy can be used to directly probe the chemical environment surrounding the sulfur/selenium sites as a function of their redox state.

2032-Pos Board B51

Comparative Characterization of Apo-, Reconstituted- and In Vivo-Folded forms of a Durum Wheat Metallothionein

Zehra Sayers[1], Mert Aydin[1], Erhan Bal[1], Filiz Yesilirmak[1], Petr V. Konarev[2], Maxim V. Petoukhov[2], Manfred Roessle[2], Alexey G. Kikhney[2], Weifeng Shang[2], Dmitri I. Svergun[2].
[1]Sabanci University, Istanbul, Turkey, [2]EMBL Hamburg Outstation, c/o Desy, Hamburg, Germany.

Durum wheat metallothionein (dMT), a plant type 1 metallothionein, with a long ''hinge'' region between metal coordinating cysteine clusters, is efficient cadmium (Cd) chelator. In this work, biophysical features of purified recombinant holo-dMT, its demetallated form (apo-dMT) and the reconstituted Cd_5-dMT are compared to obtain insight into the structure and metal binding features of this protein. Results show that the purified holo-dMT is polydisperse and has 5.3 ± 0.5 Cd^{2+} ions per molecule. Demetallation followed by size exclusion chromatography yields homogeneous apo-dMT which can be reconstituted with Cd^{2+}. Synchrotron small angle X-ray scattering (SAXS) demonstrates that apo-dMT, at pH 2.0, is flexible and extended in solution. According to UV-vis, CD and native-PAGE data conformation of apo-dMT is sensitive to pH changes in the range 2.0 to 8.0. Reconstitution of the apo-protein at pH 8.0, with Cd^{2+} appears to take place in two phases during which first the monomer is folded to accommodate 5 Cd^{2+} ions and then reorganization into oligomeric forms allows incorporation of further metal ions. SAXS data indicate that holo-dMT has limited flexibility in structure, but its conformation is significantly more compact than that of apo-dMT. Results of UV-vis and circular dichroism spectroscopy show that the in vitro folded protein is structurally different from the purified holo-dMT with the same number of Cd^{2+} ions.

This work was supported by Sabanci University Internal Research Fund Project No. IACF08-00514 and by the bilateral program TUBITAK-Julich Research Center projects TBAG-U-155 and TBAG-U-157.

2033-Pos Board B52

N-Propanol Based Solubilization Buffer Enhances Refolding Yield of Inclusion Body Protein by Populating Intermediates to the Folding Pathway

Anupam Singh, Vaibhav Upadhyay, Amulya K. Panda.
National Institute of Immunology, New Delhi, India.

Most of the times, high level expression of recombinant proteins in bacteria results in accumulation of recombinant proteins into inclusion body (IB) aggregates. To obtain native protein from these aggregates, it is necessary to solubilize these aggregates followed by refolding of solubilized protein by appropriate refolding method. Conventionally, high concentration of denaturant like urea or guanidinium chloride (GdmCl) is used for solubilization of inclusion bodies which often results into aggregation of protein during refolding process. In the present study we have evaluated a novel solubilization method using n-propanol in presence of low concentration of urea. n-Propanol based solubilization agent was compared with traditional solubilization agents like 8 M urea and 6 M GdmCl for solubilization efficiency, structure and stability of the solubilized model protein, recombinant human growth hormone (hGH). hGH IBs were found to be tough and were only solubilized efficiently in presence of high concentration of denaturants (8 M urea or 6 M GdmCl). 4 to 6 M n-propanol in combination with 2 M urea was sufficient for the efficient solubilization of hGH IBs. Aggregation during refolding was also studied and it was found that solubilization with n-propanol based buffer resulted into bioactive hGH without aggregation giving significantly higher refolding yield in comparison to those obtained with urea and GdmCl based buffers which resulted in aggregation of hGH during refolding. From the results obtained, it can be concluded that solubilization of hGH IBs in n-propanol based buffer results in a partially folded folding intermediate of hGH which readily folds into native form on dilution with reduced chances of protein getting into aggregation pathway.

2034-Pos Board B53

Structural Stability of Tandem Calponin-Homology (CH) Domains Originates from their C-Terminal CH2 Domain

Swati Bandi, Surinder Singh, **Krishna Mallela**.
Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO, USA.

Tandem CH domains form a major class of actin binding domains. In general, the N-terminal CH domain (CH1) weakly binds to F-actin whereas the C-terminal CH domain (CH2) does not bind to actin. However, when CH2 is linked to CH1, the actin binding efficiency increases by more than ten times, which implies a functional cooperativity between the two CH domains. The structural cooperativity underlying this functional cooperativity and the physical mechanism by which CH2 domain enhances the actin-binding efficiency is not understood. In this study, we examined the relative stabilities of the two CH domains of utrophin and dystrophin. The isolated CH1 domain of utrophin does not exist as a stable structure; it is more like a destabilized ''molten globule'' state. However, its CH2 domain folds to a stable structure, as evident from its alpha-helical spectrum and cooperative melt. Similar to utrophin, the CH2 domain of dystrophin is a well-structured protein, and has similar stability as that of the full-length tandem CH domain. The CH1 domain of dystrophin is quite unstable and aggregates severely. These results indicate that the CH1 domain requires CH2 for its folding, or in other words, CH2 acts as a template for CH1 folding. These stability experiments support the earlier hypothesis proposed based on cryo-EM studies that the major role of CH2 might be to stabilize the tandem CH domain. Additional support comes from the literature: no molecular structures are available for the CH1 domains alone, suggesting that they might be unstable in the absence of CH2. In contrast, structures have been determined for numerous CH2 domains. These experimental observations indicate that the CH2 domain enhances the actin-binding function by imparting structural stability to the tandem CH domains.

2035-Pos Board B54

Calcium-Induced Folding and Secretion of Alkaline Protease (Apra) from Pseudomonas Aeruginosa

Liang Zhang, Patrick H. Thibodeau.
University of Pittsburgh, Pittsburgh, PA, USA.

Alkaline protease (AP) is a known virulence factor secreted from Pseudomonas aeruginosa (Pa), which causes serious infection in patients with cystic